

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGISTRATION  
TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

CYANAZINE

Chemical Code # 1640    Tolerance # 307  
SB 950 # 057

December 2, 1987

Revised 12/28/88, 9/26/89, 7/09/90, 9/19/91, 8/13/92, 10/29/93, 6/22/94,  
2/10/95, 3/3/95, 3/29/95, 5/18/95

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect (not oncogenic)
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, possible adverse effect
Teratology, rabbit:	No data gap, no adverse effect

Gene mutation:                      No data gap, possible adverse effect

Chromosome effects:                No data gap, no adverse effect

DNA damage:                        No data gap, possible adverse effect

Neurotoxicity:                      Not required at this time

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Toxicology one-liners are attached.

All record numbers through 123811 and 968050.

\*\* indicates an acceptable study.

**Bold face** indicates a possible adverse effect.

File name: T950518

Revised by S. Morris, 08/13/92; Gee, 10/29/93, P. Iyer, 6/22/94, Gee, 2/10/95 and 3/3/95,  
Aldous, 3/29/95 and Gee, 5/18/95.

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

## COMBINED, RAT

**\*\*307-069; 091451;** "Combined Chronic Toxicity/Oncogenicity Study with Cyanazine (IN R1957) Two-Year Feeding Study in Rats", HLR # 23-90, Matthew S. Bogdanffy, Haskell Laboratory, Newark, DE., 5/11/90. Cyanazine (> 96% purity) was fed in the diet for 24 months at 0, 1, 5, 25, or 50 ppm to 62 rats (Cr1:CD\*BR) / sex / group. Ten rats / sex / group were sacrificed and necropsied at 12 months. Toxicologically significant, treatment-related effects were: reduced body weights in both sexes at 25 and 50 ppm and decreased absolute kidney weight and increases in relative testes weight, and hyperactivity in males at 50 ppm. A possible adverse effect was indicated by increased malignant mammary gland tumors in females at 5, 25, and 50 ppm. The study is acceptable (H. Green and S. Morris, 07/15/91).

NOTE: The possible adverse effect was listed under the data gap status for the rat oncogenicity test type and not the rat chronic toxicity test type on the Data Gap Status page (S. Morris, 07/16/91).

307-075; 113787: This document contains historical control data from eight studies using Cr1:CD\*BR rats on the incidence of malignant mammary tumors in females, granulocytic hyperplasia of the bone marrow in males, extramedullary hematopoiesis of the spleen in males, and demyelination of the sciatic nerve in females. No worksheet was done (S. Morris, 8/13/92).

## CHRONIC TOXICITY, RAT

NOTE: See combined rat above for a more recent, acceptable study.

307- 033, 035 through 042 38530, 38532, 38533, 38534, 38535, 38536, 38538, 38539, "Toxicity Studies on the S-Triazine herbicide Bladex (DW 3418): 2 Year Oral Experiment with Rats", (Shell Tunstall Laboratory, #TLGR0063.70, 1970), Cyanazine, batch FC5146, purity not stated; fed 2 years to 24/sex/group at 6, 12, 25 or 50 ppm; 48/sex for controls; diet analysis done; slight reduction in weight gain at 25 or 50 ppm only effect reported. NOEL = 12 ppm (decreased weight gain). No adverse effect reported. Complete, unacceptable, not upgradeable: this study was conducted well before modern study guidelines, and is thus unacceptable to DPR for many reasons. Perhaps the most important deficiency was inadequate treatment group size, and hence poor design for meaningful statistical evaluation. (Gee, 12/85: 1-liner edited by Aldous, 3/22/95).

EPA one-liner: Levels tested in Carworth Farm E. strain - 0, 6, 12, 25, and 50 ppm. Invalid.

307-012; 968046; Summary of # 038530.

307-035; 038532; Addenda to # 038528 and # 038530 - statistics & tumor analyses. (Gee, 1/3/86, worksheet completed)

307-005; 968019; Summary of # 038530.

307-032; 038528; Full report (with #968020), "Toxicity Studies on the S-Triazine Herbicide Bladex: Second 2 Year Oral Experiment in Rats", (Shell Tunstall Laboratories, #TLGR0018.73, July 1973), Cyanazine, Batch 5097, purity > 97%, fed to 24/sex/group at 0, 1, 3 or 25 ppm for 2 years; No adverse effect reported. Minor variations - not dose-related - in hematology, clinical chemistry. NOEL: 3 (or 25?) ppm. Unacceptable, not upgradeable because of many deficiencies with respect to modern guidelines. Major problems of the study included an unusually high incidence of unexplained convulsions about 3 months into the study (indicating an animal health management problem), lack of diet analysis, poorly selected dose levels (in

view of equivocal thyroid effects in the 1970 study), inadequate group sizes for oncogenicity assessment, no clear indication of the extent of the pathology examinations, inadequate clinical chemistry protocol, and a lack of individual data, except for histopathology findings in key organs. (Gee 1/3/86; 1-liner expanded by Aldous, 3/22/95).

EPA one-liner: Levels tested in Carworth Farm E. strain - 0, 1, 3, and 25 ppm. Oncogenic NOEL - inadequate data; systemic NOEL - inadequate data. Supplementary.

307-012; 968020; Summary # 038528.

307-040; 038537; Individual body weight and serum chemistry for # 38528. (Gee 1/3/86, worksheet completed)

307-035 038532 [re-examination of record entitled: "Statistical analysis of tumor data from two Bladex\* chronic studies in rats (TLGR.0063.70 and TLGR.0018.73)", Westhollow Research Center, Houston, TX. These were Record Nos. 038530, -532 to -536, and -538 to -539 for the 1970 study and Record No. 038528 for the 1973 study. Thyroid C-cell tumor data from the above two studies were examined, along with C-cell tumor data from the more recent Haskell combined rat study (Record No. 091451). Based on evaluation in the present worksheet, the data do not warrant attributing C-cell tumor incidence to treatment, despite the increase in C-cell tumors in 50 ppm males in the [invalid] 1970 Tunstall study (significant by Fisher's Exact Test, one-tailed,  $p < 0.05$ ). Aldous, 3/22/95.

#### CHRONIC TOXICITY, DOG

\*\* 307 -060, -061, 054481; "One-Year Oral Dosing Study in Dogs with the Triazine Herbicide-Cyanazine," Report No. HLA 6160-104; Hazleton Laboratories America, Inc., Madison, WI.; 12/30/86; cyanazine (98%) in the feed at 200,100, 25, 10, or 0 ppm to Marshall beagles, 6/sex/level, for 1 year; no adverse effect reported; initial body weight loss with reduced food consumption and gain thereafter documents MTD, weight of 200 ppm females 25% below

control at termination; statistically significant but toxicologically unimportant slight sporadic increases in platelet counts and inorganic phosphorus with reduced total protein, albumin and calcium; not regarded as adverse effects; overall NOEL=25 ppm (about 0.7 mg/kg/day); originally found unacceptable but possibly upgradeable with submission of test article purity and feed analysis data, (F. Martz, 11/3/87); study acceptable with data at rec. # 67641, (S. Morris, 07/08/88).

307-065; 067641: This is information supplemental to 307-060, 054481 and contains analysis of test material purity and content of animal feed.

307-032; 038529; "Toxicity Studies on the S-Triazine Herbicide Bladex (DW 3418): 2 Year Oral Experiment with Dogs", (Shell Tunstall Laboratory, #TLGR.0005.70, September 1970), Cyanazine, batch FC 5097, purity > 97%, 4/sex/group (6/sex for controls) at 0, 0.625, 1.25 or 5 mg/kg/day by capsule for 2 years; vomiting frequently at high dose; reduced liver, body weight in high dose with no histopathology data in report to evaluate. No adverse effects. NOEL: 0.625 mg/kg. Unacceptable - no consistent hematology/ clinical chemistry findings; Inadequate pathology. Not upgradeable. (Gee, 1/2/86)

307-005; 035630: Summary of # 038529.

307-012; 968021: Summary of # 038529.

#### ONCOGENICITY, RAT

See combined rat above.

#### ONCOGENICITY, MOUSE

**\*\* 307-034; 038531;** "A Two Year Feeding Study of Bladex in Mice" (Shell Sittingbourne Research Center, #SBGR.81.171, December 1981), Cyanazine (98%) fed at 10, 25, 250 or 1000 ppm for 2 years to 50/sex/group (100/sex for controls); diet analysis included. Adverse effects: increased renal cortical tubular dilation and epithelial vacuolation, myocarditis. No oncogenicity. Poor palatability in feed resulted in symptoms of low caloric intake. NOEL: 10 ppm. Acceptable. (Gee, 1/3/86) NOEL for body weight deficit changed to <10 ppm. (Gee, 2/10/95)

EPA one-liner: Carcinogenic NOEL > 1000 ppm (HDT); systemic NOEL  $\leq$  10 ppm (LDT) (decreased body weight - both sexes). Doses tested: 0, 10, 25, 1000 ppm. CD strain. Minimum.

307-012; 968045: Summary (20-page) of #38531. (Wong 3/25/85, worksheet completed)

#### REPRODUCTION, RAT

307-043; 038540; "Results of Reproduction Study of Rats fed Diets Containing SD15418 over 3 Generations", (Hine Laboratories, # M-174-69, August 1969), 3 generation with 2 litters per generation. Dietary at 0, 3, 9, 27 or 81 ppm. Very little data collected but there appears to be a slight decrease in adult terminal weight at 81 ppm. No adverse effect reported however, NOEL can not be determined from limited data. Unacceptable, Not Upgradeable. Design of study inadequate, no food consumption, no analysis of diet, limited necropsy and weight data. (Parker, 12/12/85)

EPA one-liner: NOEL > 80 ppm (HDT). Doses tested: 0, 3.9, 27, 80 ppm. Long Evans strain.

307-012; 968048: Summary of # 38540.

**\*\* 307-062; 061221;** "Two-Generation Reproduction Study of Technical Bladex\* Herbicide (SD 15418) in Rats," SRO 15-87, WIL-93001; WIL Research Laboratories, Inc., Ashland, OH; 8/12/87; cyanazine, 98%; The F0 generation consisted of 28 rats/sex/group that were exposed to dietary concentrations of 0, 25, 75, 150, or 250 ppm for  $\geq$  70 days then through 2 mating/weaning cycles of the F1a and F1b litters. The F1 generation consisted of 28 rats/sex/group randomly-selected from F1b litters and subjected to the same exposure/mating protocol as the F0's, producing the F2a and F2b litters. Adequate exposure was indicated by Z 20% decrease in adult body weight gain at 250 ppm (NOEL = 150 ppm) and dose-related decreases in pup weight gain during nursing. The significant toxicological finding was decreased pup

viability: Fla pups at 250 ppm on day 21 and F2a pups at 150 and 250 ppm on day 4 (NOEL = 75 ppm). A possible adverse effect was indicated (pup NOEL < adult NOEL). The analytical concentration was estimated to be 78.5% of the nominal value. The study was upgraded from unacceptable (Morris/Parker, 06/09/88) to acceptable (Morris, 08/15/89).

307-050; 042237; WIL Labs, Draft protocol, received 3-7-86, for Two Generation Reproduction Study of Bladex in Sprague Dawley CR1: CD(SD)BR Rats. Two litters per generation at 0, 25, 75, 150 and 250 ppm in diet. There are apparent discrepancies from EPA FIFRA guidelines (limited necropsy and histopathology). Memo written. See completed study at 307-062, 061221). (Parker, 3/21/86, no worksheet).

307-050; 042238; WIL Labs, Draft summary letter & interim report, received 3-7-86 for 2 Generation Reproduction Study (Sprague Dawley CR1: CD(SD)BR rats) (0, 25, 75, 150 and 250 ppm). These data are for the first mating of the first generation of the study at 307-062, 061221. Weight gain in adults at 150 and 250 ppm decreased. Pup survival at day 21 of lactation significantly decreased at 75, 150 and 250 ppm. Additional data on necropsy, fertility indices, and Flb survival have been requested from sponsor. Letter from Shell, 8-4-86, acknowledges this request but does not agree to submit data (307-055). (Parker, 3/21/86, worksheet completed)

307-068; 074431; This document contains additional data on the stability of the test material used in the study at doc. # 307-062, rec. # 061221. Evaluation of these data did not result in a change of study status (Morris, 08/19/89).

307-068; 074432; This document contains additional data on analysis of the diet used in the study at doc. # 307-062, rec. # 061221. These data were evaluated with doc. # 307-068, rec. # 074431. Evaluation of these data did not result in a change of study status (Morris, 08/19/89).

307-068; 074433; This document contains revised necropsy tables from the study at doc. # 307-062, rec. # 061221. Evaluation of these data resulted in a change of study status to acceptable.

#### TERATOLOGY, RAT

\*\* 307-045; 038546; "Final Report, Teratologic Evaluation of Bladex in SDCD Rats", (Research Triangle Institute, # WRC-RIR-311, 6/21/83), Cyanazine, 98.5%, given by oral gavage @ 0, 1, 3 or 30 mg/kg/day to 30/group on days 6-15 of gestation. No adverse effects. Maternal NOEL = 3.0 mg/kg/day (body weight and clinical signs); developmental NOEL = 30.0 mg/kg/day. Originally reviewed as unacceptable, upgradeable (individual animal data, dose justification, analysis of dosing formulations) (Parker 12/31/85). Analyses of dosing formulations have been supplied and are acceptable (050, 042247). Individual data have been supplied in the form of copies of workbooks and should be compiled into tables (055, 047286). Dose justification has been supplied (055, 047285). Change of status to Acceptable. (Parker, 12/4/86)

EPA one-liner: Teratogenic NOEL  $\geq$  30 mg/kg/day (HDT); maternal NOEL = 3.0 mg/kg/day; fetotoxic NOEL  $\geq$  30 mg/kg/day. Levels tested: 0, 1, 3, and 30 mg/kg/day by gavage in SD-CD rats. Minimum. Reclassified as supplementary data due to disparity in findings with the Argus study (#619-002P).

307-050; 042247: This is information supplemental to 307-045, 038546 and contains analysis of dosing formulations.

307-055; 047285: This is information supplemental to 307-045, 038546 and contains justification of dose.

307-055; 047286: This is information supplemental to 307-045, 038546 and contains individual data.

307-014; 025218: Partial (9-page) duplicate of (WRC-RIR-311) 038546. (V Van Way, no worksheet)

307-050; 042248: Addendum to 038546 - sponsor justification of dose levels, however, tables and figures missing, therefore cannot evaluate. (Parker, 4/7/86, no worksheet)

307-051; 042249: Addendum to 038546 - individual data (copies of workbooks, not compiled). (Parker, 4/7/86, no worksheet)

307-050; 042245: Supplement to 038546. Review by E.M. Johnson, 8/85, of Jefferson Medical College, on CR-CD rats. (Parker, 12/1/87, no worksheet)

**\*\* 307-045; 038544;** "Technical Bladex (SD 15418) Teratology Study in Rats", (Shell Westhollow Research Center, WRC RIR-180, WTP-78, 11-13-81) Cyanazine, 98.5%, administered by gavage to 30 Fischer 344 rats/group at 0, 1, 2.5, 10 or 25 mg/kg/day. Maternal NOEL = 2.5 mg/kg/day (minor decrease in body weight gain). Developmental NOEL = 10 mg/kg/day (an- or microphthalmia and skeletal variants seen at 25 mg/kg/day). Diaphragmatic hernia seen at a low incidence in all treated groups and not in control. EPA in PD-1 (4-10-85) considered cyanazine "teratogenic" and requested an additional study. Possible adverse effects due to unusual fetal findings in the presence of minimal maternal toxicity. Initially reviewed, Parker 12-23-85, as unacceptable (need analysis of dosing suspensions). This was supplied (052, 042250) and reviewed. Study upgraded to Acceptable. Parker, 4/8/86.

EPA one-liner: Teratogenic NOEL = 10 mg/kg/day; maternal NOEL = 2.5 mg/kg; fetotoxic NOEL  $\geq$  25 mg/kg (HDT). Doses tested: 0, 1.0, 2.5, 10.0, 25.0 mg/kg - Fischer 344 strain. Supplementary.

307-052; 042250: This is information supplemental to 307-045, 038544 which contains analysis of dosing suspensions.

307-012; 968047: Summary (9-page) of # 038544. (Wong 3/22/85, worksheet completed)

307-050; 042244: Supplement to 038544. Review by E.M.Johnson, 8/85, of Jefferson Medical College, of Fischer 344 rat study # WRC RIR-180 - questions some findings. (Parker, 12/1/87, no worksheet)

**\*\* 027 27089** "Study of the Developmental Toxicity of Technical Bladex (SD 15418) in Fischer 344 Rats" (Argus Research Laboratories 4-18-85). By gavage at 0, 5, 25 and 75 mg/kg/day on days 6-15 of gestation to 70 mated females/dose level. Fetuses were examined on gestation day 20 and pups 21 days after natural delivery. Maternal toxicity NOAEL = 5 mg/kg/day (increased incidence of clinical observations at 25 and 75 mg and 13/70 deaths at 75mg). Developmental toxicity NOEL = 5 mg/kg/day (increased number of fetuses and pups with microphthalmia or anophthalmia at 25 and 75 mg, decreased litter size and weight at 75 mg, increased total litter resorptions at 25 and 75 mg/kg, and decreased live litter size and survival to day 21 of lactation at 75 mg). Adverse effect since developmental toxicity seen at levels of cyanazine causing only slight maternal toxicity. Initially reviewed by J. Parker (11/13/85) and upgraded to acceptable status with results of analytical evaluation in 055 047289 by J. Parker (12/5/86). Change to possible adverse developmental effect based on eye defects, others, with NOEL of 5 mg/kg/day (as before) by P. Iyer (6/21/94).

307-055; 047287: This is information supplemental to 307-027, 027089 and contains analysis of dosing solutions.

307-050; 042246: Supplement to 027089. Review by E.M. Johnson, 8/85, of the Jefferson Medical College, on Fischer 344 rats. (Parker 12/1/87, no worksheet)

After re-reviewing the later rat teratology study (027089) and the maternal toxicity of the individual dams (i.e., those producing fetuses with defects), 307-045 038544 also was re-examined. The maternal toxicity (evaluated using group mean data for body weight gain) was not corroborated for the individual dams. This supports the hypothesis for a direct developmental effect rather than the malformations being a consequence of maternal toxicity (P. Iyer, 6/22/94, no worksheet).

SUMMARY: In considering the studies conducted with the Fischer 344 rat, more weight is placed on the results of the later study (307-027, 027089) since it was conducted on a larger number of animals per group and had the addition of a post natal phase. The study conducted with Sprague Dawley rats, however, did not indicate a unique hazard to the developing organism (Parker, 12/2/87). (Revised by Iyer, 6/21/94).

#### TERATOLOGY, RABBIT

\*\* 307-045; 038545; "Teratology Study in New Zealand White Rabbits Given Bladex Orally", (Shell Sittingbourne Research Centre, # SBGR.82.357, November 1982, amended 1/23/83), Cyanazine (SD15418), 98%, given by oral gavage to 22 mated NZW rabbits/group @ 0, 1.0, 2.0 or 4.0 mg/kg/day; slight maternal toxicity at 4 mg/kg/day and possibly 2 mg/kg/day (decreased food consumption & weight gain); fetal effects at 4 mg/kg include decreased litter size, increased resorptions and increased 13th ribs, and at 2 mg/kg, slight increase in resorptions and 13th ribs. No adverse effects. NOEL (maternal and developmental) = 1 mg/kg/day. Acceptable and complete (Parker, 12/24/85).

EPA one-liner: Teratogenic NOEL  $\geq$  4 mg/kg/day (HDT); maternal NOEL = 1 mg/kg (LDT); fetotoxic NOEL = 1 mg/kg (LDT). Note: At 4 mg/kg - significant increase in # dead fetuses/dam and increased incidence of anomalies, i.e. domed head in 4 fetuses (2 litters). Doses tested: 0, 1.0, 2.0, 4.0 mg/kg. New Zealand White strain. Minimum.

307-014; 025217: (11/82, Shell SGBR82.357) Partial (17 pp.) of # 038545. (V. van Way, no worksheet)

307-014; 025221: Addendum (12 pages) - list of abnormalities of # 038545. (V. van Way, no worksheet)

307-050; 042243: Supplement to 038545. Review by E.M. Johnson, Daniel Baugh Institute, 8/85. (Parker, 4/2/86, no worksheet)

307-048; 038806: "A Developmental Dermal Toxicity Study in New Zealand White (NZW) Rabbits with Bladex 4L Formulation (unaudited draft)", (WIL Research Laboratories Inc., WIL no. 93002, no date, received 12/17/85), 100% Bladex 4L, WRC 795, purity not stated, 6 hours/day dermal exposure on days 6 through 18 of gestation at 0.2, 0.6, 1.2, or 2.0 ml/kg/day with 20/level. Formulation blank control group. No adverse effects. Maternal NOEL < 0.2 ml/kg/day (decreased bodyweight gain at all treatment levels). Developmental NOEL = 0.2 ml/kg/day (decreased bodyweight and increased resorption at 0.6, 1.2, and 2.0 ml/kg/day. Unacceptable, upgradeable (need: final report (see 050 42241), results of external examination of abortuses, analysis of test material, explanation of continuous collaring of animals from August 31 through September 2, results of analysis of samples taken after washing backs of rabbits (pg. 10), explanation of correlation between abortion and food restriction (pg. 22), justification for lack of untreated control). (Parker, 12/23/85)

307-048; 038807: This document is supplemental to 038806 and contains water analysis and historical controls data.

307-049; 038808: Draft of method development pilot study, No. 93002A, conducted after main dermal study = adjunct to 038806 and supplemental to 042241. (Parker, 3/21/86, worksheet completed)

307-050; 042239: Letter 2/5/86 WIL to sponsor clarifying findings in # 038806. Does not fulfill CDFA data request on abortuses. Study remains unacceptable. (Parker, 3/21/86, worksheet completed)

307-050; 042240: Analyses for dosing solutions 6/85 for # 038806. (Parker, 3/21/86, no worksheet)

307-050; 042241: (Feb/1986, WIL Study NO.93002 & 93002A) Final report (of both the main and the subsequent method development pilot study) - no data on abortuses; study remains unacceptable. (Parker 4/7/86) See prior review of 038806 (unaudited draft). (Parker, 3/21/86, worksheet completed)

307-030; 042306: Protocol for developmental dermal toxicity study (WTP 287) # 042241. (Parker, 12/1/87, no worksheet)

\*\* 307-054; 047598; "A Dermal Developmental Toxicity Study in New Zealand White (NZW) Rabbits with the Bladex\*4L Formulation," Record No. WRC RIR-451; WIL Research Laboratories Inc., Ashland, OH; 06/17/86; Bladex\*4L, 44.7% cyanazine; by dermal exposure on days 6-18 of gestation at 0.2, 0.6, 1.2, and 2.0 ml/kg/day; formulation blank and sham controls included; maternal NOEL = 0.2 ml/kg/day (decreased weight gain and food consumption at 0.6 ml/kg/day); developmental NOEL = 0.6 ml/kg/day (lower fetal body weight); no adverse effect reported; originally found unacceptable but upgradeable with submission of test article purity and feed analysis data, Parker, 08/21/86; study acceptable with rec. # 067642; (S. Morris, 07/08/88). Note: The previous versions placed the developmental at 0.2 ml/kg/day with no justification. Gee, 3/3/95.

307-065; 067642: This is information supplemental to 307-054, 047598 and contains analysis of test material purity.

307-050; 042242: Protocol for study (WTP 344 WIL 93003) # 047598. (Parker 4/7/86, no worksheet)

SUMMARY: the oral rabbit teratology study was conducted with 98% cyanazine while the dermal teratology studies were conducted with the formulated product Bladex 4 L. When exposure assessment is done, the NOELs will need to be corrected for actual cyanazine content. Dermal absorption data have been reviewed by Worker Health and Safety Branch. Parker, 12-1-87.

In the oral study (307-045: Vol 13) defects such as small eye and soft lens (1 fetus) and thoracoschisis ( 1 fetus) were observed in fetuses of 2 different litters (dam # 412, 655) at the 4 mg/kg dose-level. These effects were noted in the presence of severe maternal toxicity. No change in NOELs (P. Iyer 6/20/94).

#### GENE MUTATION

**\*\* 307-056; 049896;** "Genetic Toxicity Assay of Bladex Herbicide: Gene Mutation Assay in Mammalian Cells in Culture, L5178Y, Mouse Lymphoma Cells." (Westhollow Research Center, 8/12/86, Project No. 61282) Cyanazine technical, lot 16-16-0-0 (no purity stated); tested with mouse lymphoma L5178Y with and without rat liver activation at 0 to  $5 \times 10^{-1}$  mg/ml (limit of solubility); duplicate cultures, two trials. **Adverse effects:** concentration dependent increase in mutation frequency with and without activation, both trials. **Acceptable.** (Gee, 11/18/86)

307-065; 067643; "An Evaluation of the Mutagenic Potential of Cyanazine," Report No. 15418/Tox-4. This report contains only registrant's comments about mutagenicity studies and was therefore not evaluated for acceptability; (S. Morris, 07/12/88).

\*\* 307-065; 067645; "Mutagenicity Testing of Cyanazine (INR-1957) in the Σαλμονελλα typhimurium Plate Incorporation Assay," HLR # 268-87; Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; 6/1/87; cyanazine, 96%, DMSO vehicle (final concentration 3.8%); 2 replicates / 2 trials of 0, 10, 50, 100, 500, 1000, or 5000 ug / plate of TA1535, TA97a, TA98, or TA100 strains, for 48 hours, with or without S-9 activation system from Aroclor\*-induced, male, Crl:CD\*BR rat livers; cytotoxicity at 5000 ug / plate; assay sensitive to positive controls; no dose-related increases in revertants for test material; no adverse effect; study acceptable; (S. Morris, 8/9/88).

\*\* 307-065; 067646; "Mutagenicity Evaluation of Cyanazine in the CHO/HPRT Assay," HLR# 747-86, MR# 4581-449; Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; 1/7/87; cyanazine, 96%, DMSO vehicle (final concentration not stated); 2 replicates / 2 trials of 0.0, 0.1, 0.7, 1.0, 1.2, or 1.4 mM / plate of 5 x 10<sup>5</sup> BH4 clone of CHO-K1 cells for 5 hours with or 18-19 hours without S-9 activation system from Aroclor\*-induced, male, Crl:CD\*BR rat livers followed by 2 subculturings and assay of 10<sup>6</sup> cells for 6-thioguanine resistant phenotype; assay sensitive to positive controls; no dose-related increases in 6-TG-resistant mutants for test material; no adverse effect; study acceptable; (S. Morris, 8/9/88).

**No record number** "Mutagenicity of the triazine herbicides atrazine, cyanazine, and simazine in *Drosophila melanogaster*" (Murnik, M. R. and C. L. Nash, in: J. Toxicology. Environ. Health 3: 691-697 (1977)) Cyanazine (80% WP, 20% inerts) at 0.01% fed to males resulted in a significant decrease in unhatched eggs demonstrating a dominant lethal effect. Although cyanazine groups either fed or injected had higher percentages of sex-linked recessive lethals, the rates were not significant. The authors state that it did not increase sex chromosome loss, breakage or nondisjunction. **Possible adverse effect.** Unacceptable. No worksheet. (J. Gee, 5/18/95)

#### CHROMOSOME EFFECTS

307-044; 038541; "Toxicity Studies on Bladex: Dominant Lethal Assay in Male Mice after a Single Oral Dose of Bladex", (Shell Tunstall Laboratory, # TLGR.0015.74, April 1974), 24 control or 12/group male mice were given 1 oral dose at 80, 160 or 320 mg/kg followed by 1 X 8 weeks of mating at 1:3; No adverse effect on mean fetal implants or mean early fetal deaths reported; % pregnancy varied in occasional treatment groups; Unacceptable - no positive control; no individual data. Not upgradeable. (Gee 1/86)

307-012; 968050: Summary of document # 307-044, record # 038541.

307-044; 038543; "Toxicity Studies with Bladex: Chromosome Studies on Bone Marrow cells of Mice after Two Daily Oral Doses of Bladex", (Shell Sittingbourne Research Centre, # TLGR.0032.74, July 1974), Cyanazine, batch 5146, no purity stated; oral gavage in DMSO to 4/sex/group/time given 50 or 100 mg/kg a.i.; water as control or cyclophosphamide, in 2 doses 24 hours apart; sac at 8 and 24 hours; analyzed approx. 100 metaphases / animal; 3 deaths in a.i. groups - no details. No adverse effects. Unacceptable, upgradeable - no clinical signs, no justification of doses, only 2 doses, and no individual animal data. (Gee, 1/86)

307-012; 035643: Summary of document # 307-044 record # 038543.

\*\* 307-065; 067647; "Iv gtrpo Evaluation of Cyanazine (INR-1957) For Chromosome Aberration in Human Lymphocytes," HLR# 328-87, MR# 4581-460; Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; 6/18/87; cyanazine, 96%, DMSO vehicle (final concentration 1%); 2 replicates / 2 trials / assay of phytohemagglutinin-stimulated, primary, human lymphocyte cultures were evaluated microscopically for chromosome aberrations after treatment with 0.0, 12.5, 25.0, 125, 250, or 350 ug/ml with or without S-9 activation system from Aroclor\*-induced rat livers; cell cycle delay seen at 250 and 350 ug/ml without activation; assay sensitive to positive controls; no dose-related chromosome aberrations reported; no adverse effect; study acceptable; (S. Morris, 8/11/88).

**No record number** "Cytogenetic effects of cyanazine and metolachlor on human lymphocytes exposed in vitro." (Roloff, B. et al., in: Mutation Research 281: 295-298 (1992))

Cyanazine, purity not given, was incubated with leukocyte-enriched plasma from 2 male and 2 female subjects. Concentrations used were 0.01, 0.1 or 1 ug/ml for 68 hours. Two hundred total cells were scored for aberrations. At 1 ug/ml, the % cells with aberrations was increased to 9.3 compared with 5.5% in the controls. The mitotic index, however, was not altered suggesting the cyanazine was not cytotoxic. The authors conclude that cyanazine was clastogenic to human cells. No individual data. Unacceptable (insufficient information for evaluation). No worksheet. (J. Gee, 5/18/95)

Summary: The two studies with human lymphocytes have conflicting results. The publication has inadequate information for a meaningful evaluation. Therefore, more weight is given to the negative study in 067647. The two publications using plants are of unknown relevance for mammalian toxicity. Gee, 5/18/95.

## Plant Studies

**No record number** "Seedling injury and chromosome aberrations induced by Bladex, Dowpon, Princep and Tenoran." (Kahlon, P. S., in: J. Tenn. Acad. Sci. 55: 17-19 (1980)) Barley seeds were soaked in 0, 250, 500 or 1000 ppm for an unspecified time. Seeds were either planted or placed on blotters. Three hundred cells in germinating root tips were examined for chromosome aberrations and seedling injury was assessed from seedling height in those planted. Cyanazine, 94%, caused chromosomal damage. Supplemental. **Possible adverse effect.** (J. Gee, 5/18/95)

**No record number.** "Cytological effects of the pesticides Posdrin and Bladex on *Tradescantia* and *Vicia faba*." (Ahmed, M. and W. F. Grant, in: Can. J. Genet. Cytol. 14: 157-165 (1972)) Bladex, 80% wettable powder, at 200, 400 or 600 ppm was used. Plant seedlings were treated for 3, 6 or 12 hours followed by a 24 hour recovery period. In addition, 25-day-old plants of *Vicia faba* were sprayed with these same concentrations. Root tips or flower buds were examined for chromosomal aberrations. Bladex produced 3.47% of chromosome aberrations in root tips compared with 0.62 in controls. Spraying plants with Bladex was lethal. **Possible adverse effect.** Supplemental data. No worksheet. (J. Gee, 5/18/95)

## DNA DAMAGE

**\*\* 307-065; 067644;** "Assessment of Cyanazine in the *In Vitro* Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes," HLR No. 347-87; cyanazine, 96%; 2 trials with 0, 1, 5, 10, 50, 100, 500, 1000, or 1450 uM (1% DMSO in all samples) applied to primary hepatocytes in the presence of [methyl-3H]-thymidine for 18 hours followed by autoradiographic analysis of label incorporation into cells fixed with ethanol:glacial acetic acid (2:1); cytotoxicity observed at  $\geq 1$  uM as evaluated by lactate dehydrogenase activity in medium; positive controls (0.1 or 1 uM 2-acetylaminofluorene) were functional; possible adverse effect indicated by increased net incorporation of label in nuclei seen at  $\geq 1$  uM cyanazine; study acceptable; (S. Morris, 07/12/88).

307-044; 038542; "Toxicity Studies with Bladex: Studies with Bladex in the Host-Mediated Assay and with Micro-Organisms In Vitro", (Shell Tunstall Laboratory, # TLGR.0034.74, July 1974), Saccharomyces cerevisiae diploid D<sub>4</sub> strain in host-mediated (male mice) or in vitro (no activation) at 160 or 320 mg/kg in vivo or up to 4 mg/ml, 4 or 24 hours, in vitro; no increase in gene conversion frequency. No adverse effects. Incomplete, unacceptable - no individual data in plates; no description of test agent purity. Not upgradeable. (Gee, 12/85)

307-012; 035642: Summary of document # 307-044, record # 038542.

307-012; 035641: Summary of document # 307-044, record # 038542.

077 123811 "Determination of Unscheduled DNA Synthesis in Rat Spermatocytes Following In Vivo Exposure to DPX-R1957-75 (Cyanazine) by Oral Gavage." (K. S. Bentley, Haskell Laboratory, No. HLR 281-93, 4/8/93) Cyanazine technical (97.3 - 98.6%) was given by gavage to 10-20 male Crl:CDTBR rats at 0 (0.5% methyl cellulose), 125, 185, 250 or 500 mg/kg body weight on five consecutive days. Groups of 3 - 5 were sacrificed at 2 and 24 hours after the fifth dose. Mid- and late-stage pachytene spermatocytes were isolated and incubated for 22 - 24 hours with labeled thymidine, fixed and unscheduled DNA synthesis determined by

autoradiography. Cyclophosphamide and methyl methanesulfonate were positive controls and were effective. There was high mortality at 500 mg/kg so that only the 2-hour sacrifice was performed. There was no evidence of UDS at any dose or sacrifice time. No analysis of dosing suspensions. Supplemental study. (Gee, 10/28/93)

No record number. "In vivo unwinding fluorimetric assay as evidence of the damage induced by fenarimol and DNOC in rat liver DNA." (Grilli, S. et al., in: J. Toxicology Environ. Health 34: 485-494 (1991)) Cyanazine, 98.5%, was given as a single intraperitoneal injection at 46.8, 70.2 or 140.3 mg/kg to male Sprague-Dawley rats. Experiment was performed in triplicate. Animals were sacrificed 24 hours after treatment. Nuclei were isolated from the liver. DNA was exposed to pH 11.6 and ethidium bromide added. Fluorescence was measured at 30, 60 and 90 minutes during the unwinding process. Percent double-stranded DNA was calculated. Results were negative for cyanazine but positive for fenarimol and DNOC. No worksheet. Unacceptable. (J. Gee, 5/18/95)

**No record number.** "In vitro cytotoxic and cell transforming activities exerted by the pesticides cyanazine, dithianon, diflubenzuron, procymidone, and vinclozolin on BALB/c 3T3 cells." (Perocco, P. et al., in: Environmental Molecular Mutagen. 21: 81-86 (1993)) Cyanazine, 99.5%, was incubated at 50 ug/ml with BALB/c 3T3 cells for 72 hours without Wistar rat liver S9 or 4 hours with S9 activation. The concentration was selected from cytotoxicity tests. Type III foci were scored. Cyanazine increased the number of foci over controls without S9. Transformation frequency was  $41.7 \times 10^{-4}$  compared with  $3.8 \times 10^{-4}$  for untreated controls. No effect was found in the presence of S9. Two types of experiment were performed - level I and level II. **Possible adverse effect.** Unacceptable. No worksheet. (J. Gee, 5/18/95)

#### NEUROTOXICITY

Not required at this time.

records reviewed

025217; 307-014  
025221; 307-014  
027089; 307-027  
035630; 307-005  
035641; 307-012  
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038807; 307-048  
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042237; 307-050  
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records reviewed

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042240; 307-050  
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047287; 307-055  
047598; 307-054  
049896; 307-056  
054481; 307-060  
061221; 307-062  
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091451; 307-069

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113787; 307-075

123811; 307-077

968019; 307-005

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968048; 307-012

968050; 307-012